Applicants acknowledge that the amendment filed May 26, 2000 has been entered, and that all previous rejections under 35 USC §§ 102 and 103 have been removed. Claims 1-30 are pending and stand rejected under 35 USC § 112, first paragraph. In addition, claims 1-18, 27 and 29 are provisionally rejected for obviousness-type double-patenting.

## I. The Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-30 are rejected under 35 USC § 112, first paragraph, as the Examiner asserts that the specification does not enable (1) hematopoietic cell expansion media *per se*, or (2) a hematopoietic cell expansion media comprising a soluble peptide with an amino acid sequence that is at least 80% identical to amino acids 28-160 of SEQ ID NO:6.

With respect to the first ground of rejection (1) in the previous paragraph, the Examiner states that the specification does enable media and cell culture methods for expanding *primitive* hematopoietic cells. However, she asserts that flt3-L does not promote the proliferation of other, non-primitive, types of hematopoietic cells and that therefore claims 1-30 are not enabled. For the legally required evidence to support this rejection, the Examiner cites Lyman *et al.*, 1993 (Applicants' own work published after filing), which describes the effect of flt-3 ligand on various selected cell populations, and Lardon *et al*, 1994, which describes how different subsets of CD34+ cells respond to TGF-β. Applicants respectfully traverse this ground of rejection.

Applicants note that it is the *claimed* invention that must be enabled. Claim 1 recites a hematopoietic cell expansion media containing flt3-L in an amount sufficient to cause hematopoietic cell expansion. Claims 3-9, 11-17, 19, 21-27 and 29 are drawn to hematopoietic cell expansion media containing flt3-L. These composition claims are drawn to media that can be used in any number of applications. However, the intended use for a composition is simply immaterial for patentability of that composition. Thus, it is error to reject these claims on the basis that some hematopoietic cells may not proliferate in the claimed media.

As for the method claims 2, 10, 18, 20, 28 and 30, claim 2 has been amended to clarify this claim thereby rendering moot this rejection. Now, all of these claims include the recitation of claim 2 which provides "A method for expanding hematopoietic cells comprising contacting the cells with flt3-L, wherein the flt3-L is in an amount sufficient to cause expansion of the hematopoietic cells." Since these claims are directed to methods of expanding hematopoietic cells that do proliferate in response to flt3-L, such claims do not encompass inoperative embodiments. As in *Angstadt*, "nobody will use them [inoperative embodiments] and the claims do not cover them." *In re Angstadt*, 190 USPQ 214, 219 (CCPA 1976). Accordingly, withdrawal of the rejection is requested.

As for the references cited by the Examiner, Applicants submit that these references do not support non-enablement of the claims. The proposition for which Lyman *et al.*, 1993 is cited—essentially, that flt3-L affects different hematopoietic cells differently—is already disclosed in the instant application at, for example, Example 11 which begins on page 42. Applicants do not understand the relevance of Lardon *et al.*, 1994 as this reference relates to TGF-β and does not even describe the use of flt3-L.

Further, enablement of a claimed invention is determined as of the *filing date*. Thus, references published after the effective filing are not available to support an enablement rejection under § 112: *In re Hogan and Banks*, 194 USPQ 527, 536 (CCPA 1977); *accord, United States Steel Corp. v. Phillips Petroleum Co.*, 9 USPQ2d 1461, 1465 (Fed. Cir. 1989). In *In re Hogan*, the Examiner and the Board asserted that the claims at issue were non-enabling for other species at issue and, hence, the scope of enablement was insufficient to support the breadth of the claims. *Id.* As evidence in support of this rejection, the Examiner and Board cited several references published *after* the effective priority date. *Id.* The CCPA in *Hogan* reversed the rejection because the Examiner and Board relied upon these later filed references. *Id.*

However, what was found impermissible by the Court in *Hogan* is precisely what has been done here. The references cited by the Examiner, Lyman *et al*, 1993 and Larden *et al.*, 1994, were published after the effective priority date of the instant claims. Accordingly, these references are not available to support the grounds for rejection of the claims under 35 USC § 112, first paragraph.

In view of the above discussion, Applicants request reconsideration and withdrawal of this rejection of claims 1-30.

With respect to the second ground of rejection (2) referenced in the first paragraph of this section, the Examiner states that the specification teaches that amino acids 28-160 of SEQ ID NO:6 has the same biological activity as flt3-L, but that the specification does not enable media and methods of culturing hematopoietic cells wherein the media comprises soluble polypeptide that comprises an amino acid sequence that is at least 80% identical to the amino acids 28-160 of SEQ ID NO:6. The Examiner states that the specification does not disclose which amino acids in the sequence can be altered such that the flt3-L variant maintains biological activity (capable of binding receptor), and that a sequence search of the prior art and patent literature did not reveal any amino acid sequences of flt3-L having at least 80% identity to flt3-L. Therefore, the Examiner asserts that one of skilled in the art could not identify which flt3-L polypeptides that are at least 80% identical to a native flt3-L amino acid sequence would have hematopoietic expansion capabilities without undue experimentation, and rejects claims 19-26, 29 and 30. Applicants respectfully traverse.

Applicants submit that one skilled in the art could, without undue experimentation, identify flt3-L polypeptides that are at least 80% identical to a native flt3-L amino acid sequence and which bind flt3. In addition, it is not necessary that an applicant disclose all the embodiments of his invention. *In re Angstadt*, 190 USPQ 214, 218 (CCPA 1976).

Variant DNAs that encode altered flt3-L polypeptides which are at least 80% identical to amino acids 28-160 of SEQ ID NO:6 can be produced by well-known procedures such as chemical synthesis, deletion, and mutagenesis techniques disclosed on page 12, line 29, to page 13, line 34, of the present specification. The teachings of the present specification, especially when taken in combination with the knowledge available in the pertinent art, enable the skilled artisan to make mutant/variant flt3-L polypeptides without undue experimentation. The question then becomes whether testing the encoded polypeptides for the ability to bind flt3 involves undue experimentation.

A variant flt3-L polypeptide may be tested for the ability to bind flt3-L using conventional binding assays. Examples of suitable binding assay procedures are described in the present specification, *e.g.*, from the third full paragraph on page 16 through the first full paragraph on page 17, and the last two paragraphs on page 18.

In *Ex parte Mark*, 12 USPQ 2d 1904, the examiner's position was that the claims encompassed innumerable muteins, while only a limited number of successful embodiments had been shown. The Examiner further asserted that undue experimentation would be required to generate the muteins encompassed by the claim using site specific mutagenesis, and to test the resulting muteins for biological activity. In reversing the Examiner, the Board noted that "When it is considered that the claims . . . all require that the mutein produced retain the biological activity of the native protein, we consider the disclosure of this application to be enabling . . . . The record before us establishes that for a given protein having cysteine residues, one skilled in the art would be able to routinely determine whether deletion or replacement of the cysteine residues would result in a mutein which is within the claims on appeal." *Ex parte Mark*, 12 USPQ 2d 1904, 1906-1907 (BPAI 1989).

Applicants respectfully submit that generation and testing of variant flt3-L polypeptides that are at least 80% identical to amino acids 28-160 of SEQ ID NO:6 as recited in claims 19-26, 29 and 30 requires no more than routine experimentation. No evidence of record indicates that binding assays to detect binding of a flt3-L to flt3, or proliferation assays in flt3-L responsive cells, are any more than conventional, well known procedures that can be conducted by the skilled artisan without undue experimentation.

If this rejection is maintained, Applicants respectfully request that the Examiner provide reasons or evidence indicating why the testing of proteins for the ability to bind flt3-L would require undue experimentation. Applicants submit that by providing the amino acid sequence of flt3-L, they have provided all the information necessary to enable the skilled

artisan to identify variants and fragments of that amino acid sequence whose use is encompassed by claims 19-26, 29 and 30, without undue experimentation.

In view of the above, Applicants respectfully request that the rejection under 35 USC § 112, first paragraph, be reconsidered and withdrawn.

## II. Obviousness-Type Double Patenting

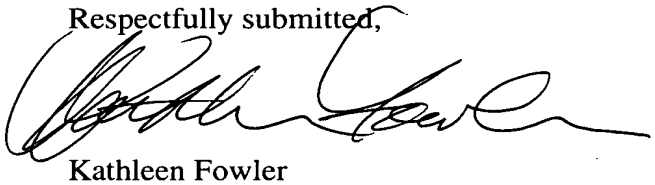
Claims 1-18, 27 and 29 are provisionally rejected under the doctrine of obviousness-type-double patenting as being unpatentable over claims 1-7, 9 and 10 of copending Application No. 08/399,404. The Examiner asserts that the "conflicting claims are not patentably distinct from each other because the claims of Application No. 08/399,404 are directed to a kit which comprises a cellular growth medium and a growth factor. Since this rejection is a provisional rejection, Applicants request that this rejection be held in abeyance until the finding of allowable subject matter.

Claims 1-18, 27 and 29 are provisionally rejected over claims 59-65 of Application No. 08/209,502. Application No. 08/209,502 is abandoned. Hence, this rejection is in error and should be withdrawn.

## Conclusion

Applicants request an early and favorable response. If the Examiner believes that any issues outstanding could be resolved by way of a telephone conference, Applicants invite the Examiner to telephone the undersigned at (206) 470-4847.

Respectfully submitted,



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**Amended Claim; Marked-up Version**

2. (twice amended) A method for expanding hematopoietic cells comprising contacting the cells with flt3-L, wherein the flt3-L is in an amount sufficient to cause [hematopoietic cell] expansion <u>of the hematopoietic cells</u>.

bj122301 11/14/00

TABLE I

Effect of Flt3-L and IL-7 on Proliferation of AA4.1+ Fetal Liver Cells.

| | Factor | | | |
|---|---|---|---|---|
| | Control | flt3-L | IL-7 | flt3-L + IL-7 |
| [$^3$H]-thymidine incorporation (CPM) | 100 | 1000 | 100 | 4200 |

The combination of flt3-L and IL-7 produced a response that was approximately four-fold greater than flt3-L alone and approximately 40-fold greater than IL-7 alone.

TABLE II

Effect of Flt3-L and IL-3 on Proliferation of C-*kit*+ Cells.

| | Factor | | | |
|---|---|---|---|---|
| | Control (vector alone) | flt3-L | IL-3 | flt3-L + IL-3 |
| [$^3$H]-thymidine incorporation (CPM) | 100 | 1800 | 3000 | 9100 |

Culture supernatant from CV1/EBNA cells transfected with flt3-L cDNA stimulated the proliferation of c-*kit*$^+$ stem cells approximately 18-fold greater than the culture supernatant of CV1/EBNA cells transfected with the expression vector alone. Addition of IL-3 to flt3-L containing supernatant showed a synergistic effect, with approximately twice the degree of proliferation observed than would be expected if the effects were additive.

## EXAMPLE 8

## Construction of Flt3-L:Fc Fusion Protein

This example describes a methof for constructing a fusion protein comprising an extracellular region of the flt3-L and the Fc domain of a human immunoglobulin. The methods are essentially the same as those described in Example 1 for construction of a flt3:Fc fusion protein.

TABLE IV

Effect of flt3-L Overexpression in Transgenic Mice

Insert TAB

| | Percentage of Positive Cells | | | |
|---|---|---|---|---|
| Marker | Unrelated Control | Littermate Control | Transgenic #1 | Transgenic #2 |
| B220 | 30.64 | 27.17 | 45.84 | 48.78 |
| sIgM | 3.54 | 2.41 | 1.94 | 1.14 |
| S7(CD43) | 54.43 | 45.44 | 46.11 | 50.59 |
| SCA-1 | 10.92 | 11.74 | 19.45 | 27.37 |
| CD4 | 6.94 | 8.72 | 12.21 | 14.05 |
| Mac-1 | 36.80 | 27.15 | 21.39 | 18.63 |

Insert TAB

The above data indicate that flt3-L overexpression in mice leads to an increase in the number of B cells, as indicated by the increase $B220^+$ cells and $SCA\text{-}1^+$ cells. Analysis of $B220^+$ cells by FACS indicated an increase in proB cells ($HSA^-$, $S7^+$). The increase in $CD4^+$ cells indicated an approximate two-fold increase in T cells and stem cells. The decrease in cells having the sIgM marker indicated that flt3-L does not stimulate proliferation of mature B cells. These data indicate that flt3-L increases cells with a stem cell, T cell or an early B cell phenotype, and does not stimulate proliferation of mature B cells or macrophages.

## EXAMPLE 12

## Analysis of the Thymus From Flt3-L-Over-expressing Mice

This Example describes the analysis of the thymus from the transgenic mice generated according to the procedure of Example 10. Six adult mice, each approximately three months of age, were sacrificed. The thymus from each mouse was removed and a single cell suspension was made.

FACS analysis demonstrated that no total change in cell number occurred and that the mice showed no change in the ratios of maturing thymocytes using the markers: CD4 vs. CD8; CD3 vs. αßTCR (T cell receptor); and CD3 vs. γδTCR (T cell receptor). However, a change in the ratios of certain cell types within the $CD4^-$ and $CD8^-$ compartment (i.e., the earliest cells with respect to development; which represent approximately 2% to 3% of total thymus cells) occurred. Specifically, $CD4^-$ and $CD8^-$ cells in the thymus develop in three stages. Stage 1 represents cells having the $Pgp\text{-}1^{++}$, $HSA^+$ and IL-2 receptor-negative ("IL-$2R^-$") markers. After stage 1, thymic cells develop to stage 2 consisting of cells having $Pgp\text{-}1^+$, $HSA^{++}$, and IL-$2R^{++}$ markers,